Plasma Disappearance Rate of Injected Human Insulin in Juvenile Diabetic, Maturity-onset Diabetic and Nondiabetic Subjects

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SUMMARY

The half-life in plasma of immunoreactive insulin was determined after the injection of eight units of unlabeled human insulin. Six juvenile diabetics and nine maturity-onset diabetics as well as eight and six nondiabetics of comparable ages were investigated. The average T½ for the groups was respectively: 10.0 ± 2.8; 12.9 ± 1.7; 10.6 ± 1.9 and 13.0 ± 2.5 minutes. There is no tendency to any difference in T½ between diabetics and nondiabetics of comparable age. However, the T½ in the older group is significantly longer than T½ in the younger age group.

Evidence from in vitro experiments, as well as from the literature, is presented which points to the fact that iodinated insulin cannot be used for the evaluation of the disappearance rate or secretion rate of endogenous insulin. Furthermore, it is emphasized that protein precipitation with TCA or Na_2SO_4 cannot be employed for the isolation of undestroyed iodinated insulin. DIABETES *18*:653-59, October, 1969.

In 1956 Berson and coworkers[1] reported their results on a study of I-131 insulin metabolism in human subjects. The half-life of labeled insulin in plasma was found to vary between twenty-two and fifty-four minutes, and no difference between diabetics and nondiabetics was observed. Evidence is accumulating, however, that iodinated insulin is metabolized in a way different from that of native insulin. In 1966 Orskov and Juel Christensen[2] and Samols and Marks[3] found a half-life of about ten minutes for injected unlabeled human insulin and endogenous insulin, respectively. This much shorter half-life has now been confirmed by other authors.[4-6]

The present study reports the disappearance rates of injected unlabeled insulin in young and old nondiabetic subjects as well as in patients with juvenile and maturity-onset types of diabetes. In addition, a few in vitro experiments will be described concerning the difference between the values of insulin half-life obtained with the use of labeled and native insulin.

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PATIENTS

The control subjects consisted of eight healthy medical students and six elderly patients admitted for nonendocrine and nonmetabolic disease. These subjects were selected so they were comparable to the diabetics regarding age, sex and weight. None of them had symptoms suggestive of diabetes mellitus and their twenty-four-hour urine specimens were free of glucose. There were six juvenile diabetics and nine elderly, maturity-onset diabetics. They were all admitted with diabetic symptoms and elevated fasting blood sugar. In all cases the diabetes was newly discovered and untreated. The pertinent clinical data of all subjects appear in table 1. We also had the opportunity to study a fifty-six-year-old patient shortly after pancreatectomy. He was operated on because of a long-standing pancreatitis and did not develop diabetes until after the operation.

PROCEDURE

After an overnight fast, eight units of human insulin (NOVO) were injected intravenously. Arterial blood samples (and in some cases also venous blood samples) were thereafter collected at three to five-minute intervals for forty-five minutes, when basal plasma insulin levels had consistently been reached. In a few cases insulin injections were repeated and blood samples were again collected.

METHODS

Serum insulin was determined by a chromatographic method.[7] In some experiments the variations in arterial blood glucose were followed employing a glucose oxidase method.[8]

TABLE 1

Clinical data of all subjects and laboratory findings

Young Subjects

	Age years	Sex	Body weight kg.	Fasting insulin conc. μU./ml.	T ½- min.	T ½- sub. min.	T ½- initial min.	Time before linearity min.
Nondiabetics								
KR	23	m	70	15	12	8	4	18
PK	24	m	70	19	12	8	3	21
EB	23	f	56	11	7	6	3	18
ES	23	m	70	23	12	8	3	21
CO	24	m	65	13	10	7	2	21
AG	25	m	54	16	9	7	2	21
MU	23	m	72	17	12	8	3	21
MB	24	f	61	14	11	9	3	24
Mean ±	24		65	16.0	10.6	7.6	2.9	20.6
S.D.M.	0.8		7	3.7	1.9	0.9	0.7	1.9
Diabetics								
EB	28	m	72	11	7	6	1	13
GS	27	f	47	5	11	6	4	24
HL	16	m	49	22	8	7	2	18
LH	19	f	54	24	8	7	3	15
FY	29	m	70	18	12	7	3	21
PA	24	m	62	4	14	11	4	24
Mean ±	24		59	14.0	10.0	7.3	2.8	19.2
S.D.M.	5		11	8.6	2.8	1.9	1.2	4.6

Old Subjects

	Age years	Sex	Body weight kg.	Height cm.	Ideal weight per cent	Fasting insulin conc. μU./ml.	T ½ min.	T ½- sub. min.	T ½- initial min.	Time before linearity min.
Nondiabetics										
GJ	59	f	53	162	83	12	9	7	3	15
VE	70	m	57	173	80	18	16	11	4	25
AF	56	f	75	167	112	10	11	9	3	20
CL	53	m	88	174	122	13	14	9	3	20
EL	55	f	76	158	125	11	14	8	5	25
PE	62	f	87	169	126	9	14	10	3	17
Mean ±	59		73	167	108	12.2	13.0	9.0	3.5	20.3
S.D.M.	6		15	6	21	3.2	2.5	1.4	0.9	4.1
Diabetics										
ES	62	f	47	166	70	16	13	10	5	24
FU	62	f	77	162	120	25	11	9	3	18
KM	56	f	71	154	120	20	12	7	3	23
MA	66	f	56	155	95	9	10	9		21
VM	69	f	66	157	108	11	15	14		18
KF	64	m	65	172	92	18	13	12	1	18
AF	68	m	79	185	100	25	13	7	3	18
RS	61	m	80	174	114	23	15	10	3	22
LI	67	f	75	166	119	18	14	9	4	22
Mean ±	64		68	166	104	18.3	12.9	9.7	3.1	20.4
S.D.M.	4		11	10	17	5.7	1.7	2.2	1.2	2.4

CALCULATIONS

Four different calculations were made on the basis of the individual semilog-scale curves (see figure 1):

a) The half-life obtained from the linear part of the graph occurring after approximately twenty minutes.

b) The time before the graph became linear.

c) The initial half-life obtained after retropolation and subtraction of the linear part of the curve from

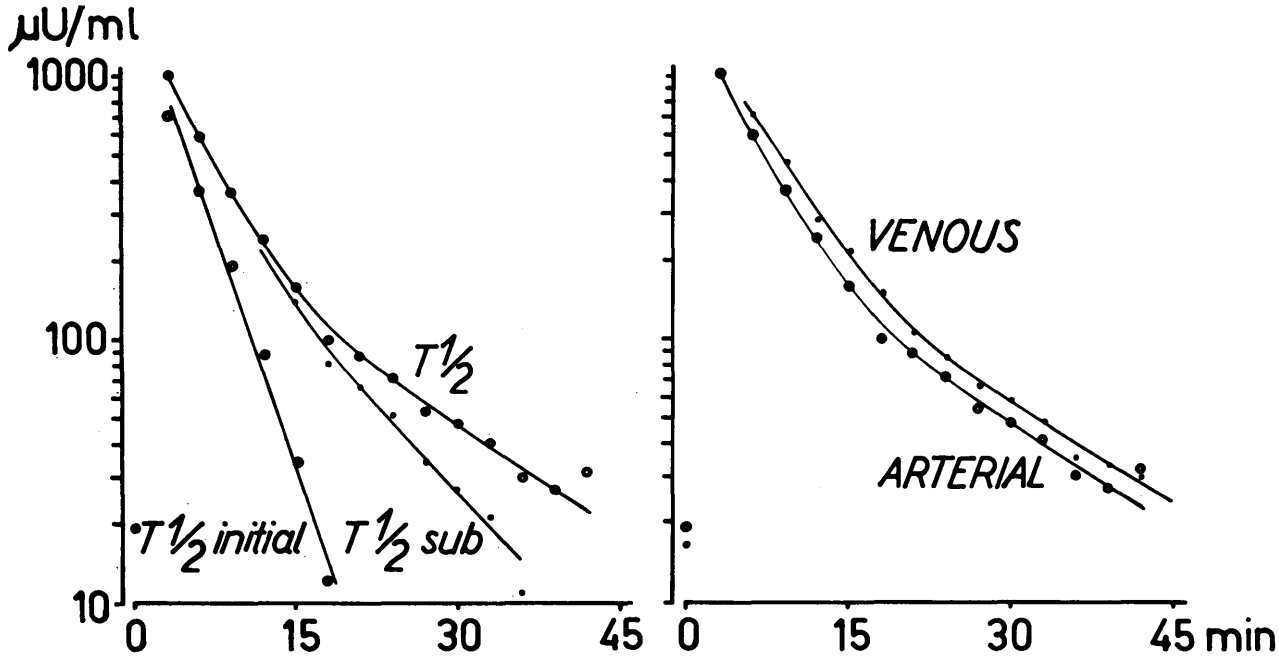


FIG. 1. Left: Disappearance rate (T½, T½-sub. and T½-initial, see text) in arterial plasma in a young nondiabetic subject (PK). Fasting plasma insulin is 19 μU./ml. Right: Disappearance rates (T½) in arterial as well as venous plasma from the same subject.

the initial steep part.

d) The half-life calculated on the basis of the linear part of the curve as in (a), but in this instance *after* subtraction of fasting concentrations.

IN VITRO EXPERIMENTS

Human I-125 insulin was incubated in human plasma at 37° C. for forty-eight hours. Four different methods were employed for the determination of undestroyed insulin: (1) TCA-precipitation, (2) 25 per cent sodium sulphate precipitation after the addition of surplus amounts of anti-insulin, (3) identification by chromatography, and (4) immunoprecipitation by the technic of Skom and Talmage.[9]

RESULTS

Figure 1 illustrates arterial as well as venous plasma insulin concentrations obtained in the first three quarters of an hour following insulin injection. The venous concentrations exceeded the arterial values throughout the experiment, the time difference between identical values being two to three minutes. This lag period is due to the time taken for the insulin molecules to be transported intravascularly as well as extravascularly from the arterial to the venous side. This was found in all of the five experiments where simultaneous arterial and venous samples were collected. The half-lives are identical, however, whether they are calculated from arterial or venous samples. Figure 2 shows another curve from the group of young nondiabetics.

The half-lives calculated from all thirty-one experiments appear in table 1. There is no difference between the results obtained in *diabetics* and *nondiabetics* in either age group. However, the T½ values are significantly greater in the two older than in the two younger age groups ($p < 0.01$). The same conclusions are reached when the half-life is calculated after subtracting the fasting concentrations (T½-sub), i.e. the older groups differ significantly from the younger ones ($p < 0.01$), while no difference obtains between normals and diabetics in either age group. The calculated initial half-life is about three minutes, but it should be noted that the initial part of the curves often had to be constructed from only two or three points. The time of distribution ranges between thirteen to twenty-five minutes. In neither of the two last-mentioned parameters were any significant differences, or tendencies to differences, observed between diabetics and nondiabetics and no correlation with age was established.

Figure 3 shows results from the two experiments where insulin was injected more than once. These maturity-onset diabetics did not become hypoglycemic after

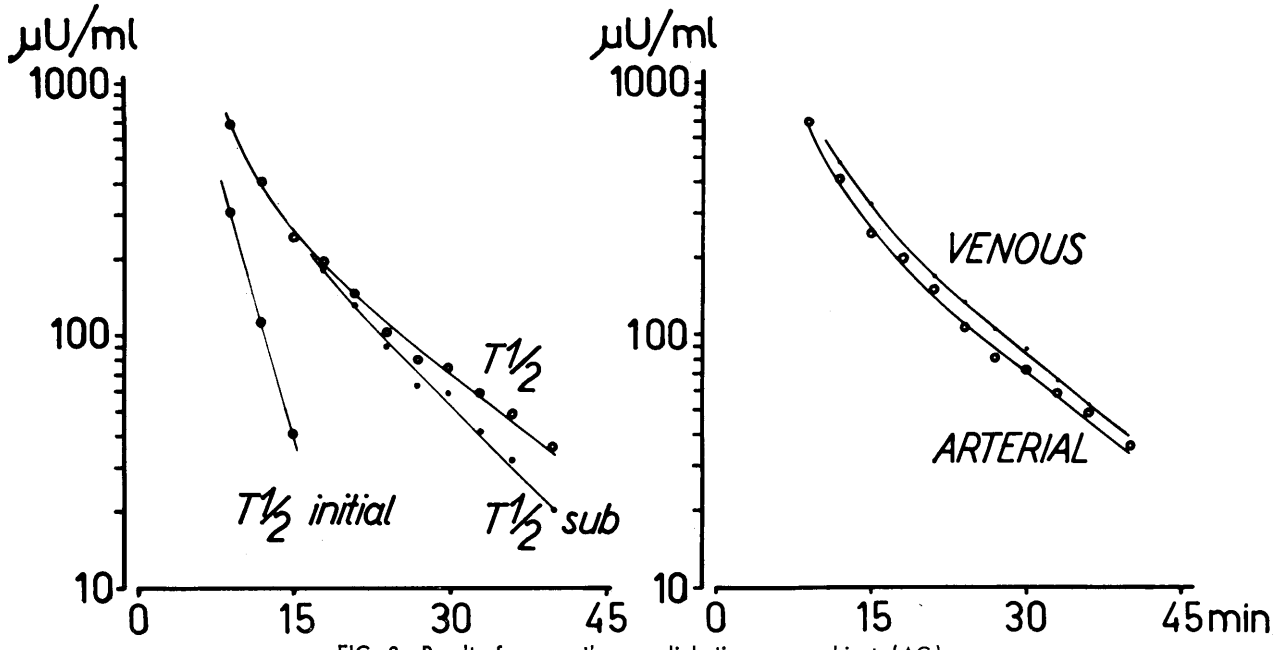
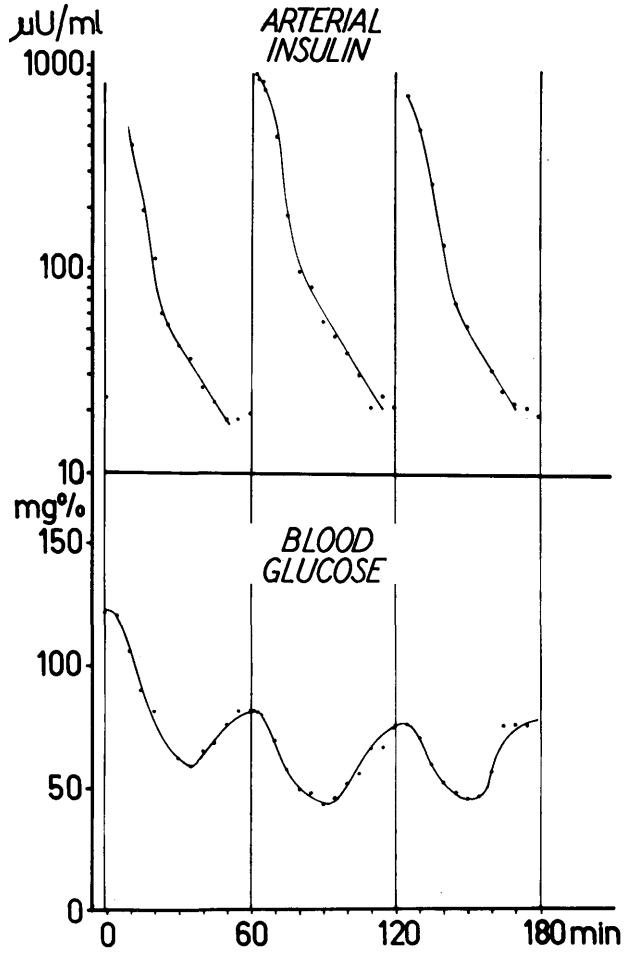


FIG. 2. Results from another nondiabetic young subject (AG).



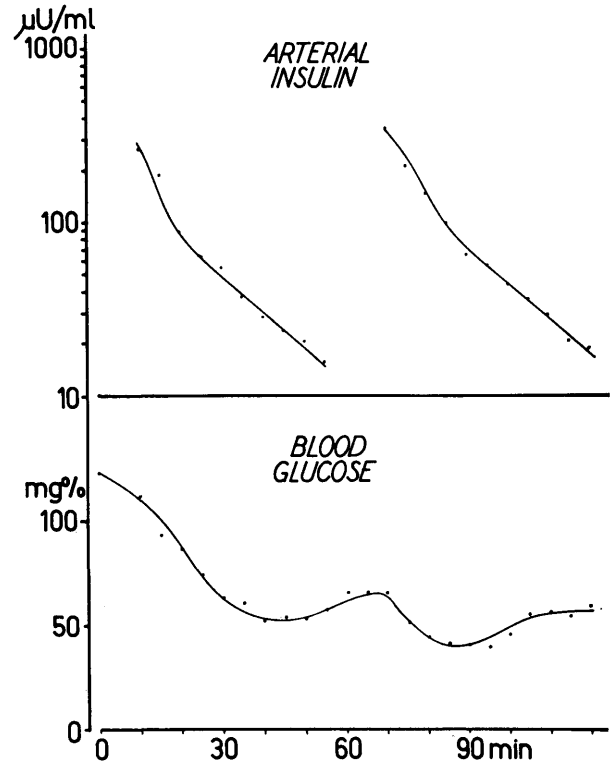


FIG. 3. Disappearance rates and blood glucose levels obtained in two maturity-onset diabetics (RS and LI), where insulin was injected (3a, left) three times at 0, 60 and 120 min. (RS) and twice (3b, above) at 0 and 60 min. (LI).

the first insulin injection. After the following injection, the blood sugar levels fell to similar values to those obtained in nondiabetics. It appears that disappearance rates are not altered during hypoglycemia, the calculated half-lives being fifteen, fifteen-and-one-half and fifteen minutes in one experiment and fourteen and fourteen minutes in the other. Finally, figure 4 illustrates the results from the fifty-year-old pancreatectomized subject; the half-life in this case was eleven-and-one-half minutes.

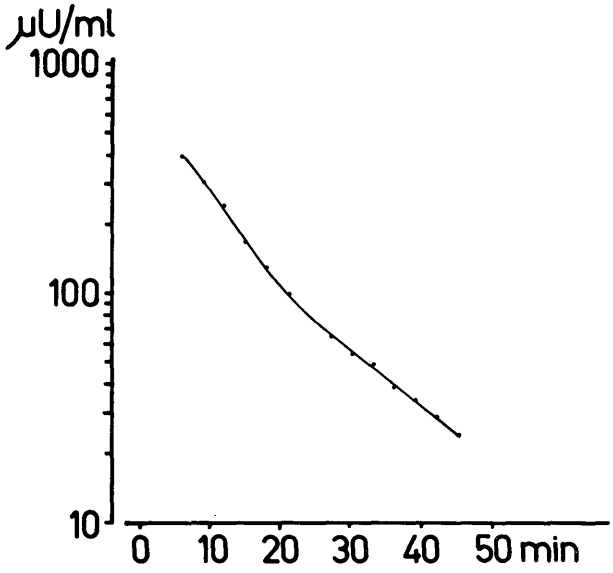


FIG. 4. Arterial plasma disappearance rate in the male pancreatectomized patient. The fasting plasma insulin is zero.

The results from the in vitro experiments appear in figure 5. It is seen that the percentages of undestroyed insulin using chromatography and specific immunoprecipitation of Skom and Talmage give very nearly identical denaturation rates. The determinations obtained with TCA-precipitation result in an apparently very much slower insulin breakdown. With 25 per cent sodium sulphate for "immunoprecipitation," the curve lies between that of the TCA-curve and the two other curves.

DISCUSSION

The half-life of unlabeled insulin measured immunologically in plasma of normals is about ten minutes. This has been demonstrated in a number of studies where different doses and different species of insulin were used. In the present investigation and in our previous study *eight units* of *human* insulin were used.

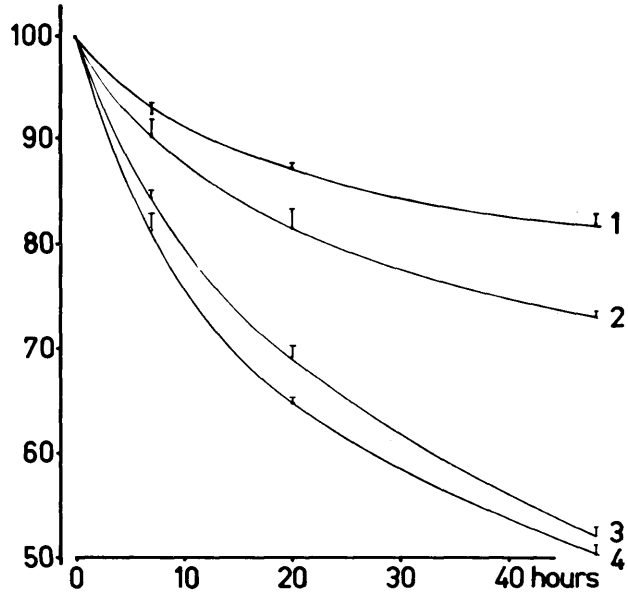


FIG. 5. In vitro experiments. Percentage-degradation of I-125-insulin in plasma during forty-eight hours. The experiment was performed on one plasma sample. After incubation for seven, twenty and forty-eight hours the process was followed by (1) TCA-precipitation, (2) Na_2SO_4-precipitation of anti-insulin complexed to the I-125-insulin, (3) paper chromatography, and (4) double immunoprecipitation a. m. Skom and Talmage. (+ 2 S.D.M., n = 3).

Samols and Marks[3] found similar values following the fall in the *endogenous* insulin level after discontinuation of glucose-glucagon infusions. These findings were later confirmed by Tomasi and coworkers[5] who injected *two units* of *pork* insulin. Rasio and coworkers[4] also found values in the same range studying the disappearance rates in six nephrectomized, exceptionally strictly regulated (hemodialyzed) patients using 0.1 *unit* of *pork* insulin per kilo. Horton and coworkers[6] used 0.1 *unit* of *beef* insulin per kilo and found an average half-life of thirteen minutes in normals and a longer average half-life in uremic subjects.

In the present study as well as in the studies quoted above, all of which were performed using immunological technics, it was found that intravenously injected insulin disappears initially at a rapid rate followed by a slower, *approximately linear,* decrease in a semilog system, beginning after about twenty minutes.

The difference observed between Berson and coworkers,[1] longer half-life using iodinated beef insulin and the shorter one found after injection of unlabeled insulin, is probably due to an impaired utilization of insulin which contains iodine atoms or of slightly de-

natured insulin molecules produced during iodination. The difference cannot be caused by the use of insulin from different species, as studies with unlabeled human, pork or beef insulins have given approximately identical disappearance rates. The theoretical and methodological studies made by Berson and coworkers[1] in their original work showed beyond doubt that neither TCA nor 25 per cent sodium sulphate could be used for the isolation of undestroyed insulin, as large amounts of protein-bound products of damage or denaturation will be coprecipitated. Paper chromatography, however, to a remarkable degree identifies insulin, which has preserved its immunological integrity; the same conclusions may be drawn from our in vitro experiments. Studies on animals by Izzo and coworkers,[10] and later by Arquilla and coworkers,[11] suggest also that the biological activity as well as the rate of breakdown of insulin is diminished after iodination. In this connection it is relevant to note that Yalow and Berson[12] a decade ago demonstrated that the protein-precipitable radioactivity showed a four-fold variation with different lots of I-131 insulin when measured one hour after administration.

It should be mentioned that Martin and coworkers[13] and Stimmler[14] have reported T½ values of approximately three to five minutes. These authors used, however, the initial steep part of the degradation curves for their calculations. They emphasize, correctly, that the disappearance of insulin in this phase is largely due to distribution.

Using the exponential fall of the plasma insulin concentrations occurring after about twenty minutes, it appears that the half-life of insulin is always approximately ten minutes. This order of magnitude obtains whether human (exogenous or endogenous), pork or beef insulins are used in small as well as in larger doses.[2-6]

To what extent does this value represent whole-body utilization of insulin? The answer to this difficult question is further complicated in studies where *unlabeled* insulin is injected, as it cannot be distinguished from the endogenously produced insulin during the experiment. We do not know what happens to the liberation of pancreatic insulin after the injection of a large amount of exogenous insulin, i.e. whether it is suppressed or not. The experiments by Frerichs and coworkers,[15] using epididymal fat pad determinations of insulin efflux from the isolated rat pancreas after exceedingly high concentrations of insulin had been added to the buffer medium, constitute the only available evidence for a negative feedback mechanism of insulin production or secretion.

If the basal liberation of insulin is not altered after insulin injection, it is reasonable to subtract the fasting insulin concentrations from the concentrations observed during the experiments. We have called the half-life calculated in this way T½-sub. These values average 7.6 minutes in young normals (table 1). If, on the other hand, insulin secretion is completely blocked, we directly measure the disappearance of *injected* insulin and obtain an average half-life of 10.6 minutes (table 1). Although this difference is relatively small, it is of interest to try and see whether insulin secretion can be suppressed or not.

Studying a totally pancreatectomized patient, whose plasma insulin level was consistently zero, we found a T½ value of 11.5 minutes. When this single value is compared to the average value of 13.0 for T½ and 9.0 for T½-sub in the six nondiabetic elderly patients, it obviously does not constitute evidence for or against this question. Hypoglycemia (hyperadrenalinemia) seemed to be of small signifcance as judged from our experiments where insulin was given repeatedly. As the values for T½ obtained before and during hypoglycemia are identical, these experiments do not tell us whether it is more reasonable to subtract fasting values or to use the direct determinations; they tell us, however, that it is permissible to compare diabetics and nondiabetics with their different blood sugar levels during the experiments.

These considerations only cover a small part of the problem: to what extent do plasma insulin disappearance rates, obtained as described here, represent whole-body utilization?

The plasma disappearance curve is the result of several factors which influence plasma insulin concentrations: (1) transport of insulin from plasma to the extracellular volume, (2) re-entry from the extracellular space to plasma, and finally, (3) irreversible loss from pools caused by degradation. For the moment the plasma disappearance rate evaluated on the basis of immunologically determined insulin concentrations after single injections of insulin seems to be our best estimate of insulin utilization, as these procedures will distinguish adequately between any *major* differences in two groups such as diabetics and nondiabetics. This point of view is supported for one thing by the finding of significant differences between disappearance rates in normals and uremic subjects.[6]

A *theoretically* more satisfactory approach is the mea-

surement of irreversible loss rates of insulin employing the estimated area under the specific activity time-curves as used by Stern and coworkers.[16] These authors, however, used *iodinated* insulin which, as mentioned above, seems to have quite a different rate of degradation to unlabeled insulin. Furthermore, they employed sodium sulphate for the precipitation of the I-131-insulin complexed to anti-insulin. It can be seen from one of Berson and coworkers' papers,[1] and from our in vitro studies, that sodium sulphate precipitates large amounts of protein-bound radioactivity derived from degradation products. For these reasons we do not believe that the insulin secretion rates reported by these authors are valid. Similar studies using tritium labeled insulin and specific immunoprecipitation or chromatography may, however, be the ideal way for the estimation of true secretion rates, always with the reservation, of course, that this, as any other practical assay of utilization, determines the systemic secretion rate, i.e. after insulin has escaped its first passage through the liver.

Until now only three other groups have studied disappearance rates of unlabeled insulin in both nondiabetics and diabetics. Tomasi and coworkers[5] studied six normals and five untreated patients with mild adult diabetes and found average T½ values of 8.1 and 7.4 minutes respectively; the difference was not significant. Results from two other groups of investigators, both using the *initial steep part* of the curves, have been contradictory. Stimmler[14] found significantly lower rates of disappearance in diabetics. The average T½ values were 4.7 as against 3.2 minutes in the normals. The mean age of the diabetics was, however, fifty-six ± nine years against forty-one ± sixteen years in the controls, so the observed difference may be due to age, although the author found no *significant* correlation with age *within* each group. Martin and coworkers[13] found no difference between diabetics and controls: the average half-life in seven normals was 4.8 minutes and in four diabetics 5.2 minutes.

In the present study, comprising six juvenile diabetics as well as nine mild adult diabetics and fourteen comparable nondiabetics, no significant difference was found and no tendency to any difference. This was the case for both age groups for the directly calculated T½ as well as for the value obtained after subtraction of fasting concentrations. However, an age difference was observed. When the two older age groups were compared with the two younger it appeared that the insulin disappearance rate was significantly lower in the older subjects.

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